Original Research Paper

LargeDifferences in Adiponectin Levels Have No Clear Effect on Multiple Sclerosis Risk: A Mendelian Randomization Study

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Abstract

Background: Mendelian randomization (MR) studies have demonstrated strong support for an association between genetically increased body mass index and risk of MS. The adipokine adiponectin may be a potential mechanism linking body mass to risk of MS. Objective: To evaluate whether genetically-increased adiponectin levels influence risk of MS. Methods: Using genome-wide significant single nucleotide polymorphisms (SNPs) for adiponectin, we undertook an MR study to estimate the effect of adiponectin on MS. This method prevents bias due to reverse causation and minimizes bias due to confounding. Sensitivity analyses were performed to evaluate the assumptions of MR. Results: MR analyses did not support a role for genetically-elevated adiponectin in risk of MS (OR = 0.93 per unit increase in natural-log-transformed adiponectin, equivalent to a two-standard deviation increase in adiponectin on the absolute scale; 95% CI: 0.66-1.33; p = 0.61). Further MR analysis suggested that genetic variation at the adiponectin gene, which influences adiponectin level, does not impact MS risk. Sensitivity analyses, including MR-Egger regression, suggested no bias due to pleiotropy. Conclusion: Lifelong genetically-increased adiponectin levels in humans have no clear effect on risk of MS. Other biological factors driving the association between body mass and MS should be investigated.

Introduction

Multiple sclerosis is the most common chronic inflammatory disease of the central nervous system (1), affecting an estimated 2.3 million people worldwide (2). MS is

thought to be initiated by T-cells which target self-antigens in the CNS, resulting in demyelination and progressive neuroaxonal injury and degeneration (1). Disease onset usually occurs in early adulthood, and prognosis is variable; however, the disease is often progressively debilitating (3).

Both genetic and environmental factors have been implicated in the etiology of MS (4). Genetic risk profiles in individuals with MS are often complex, and many non-genetic factors have been associated with the disease (4), including body weight. High BMI during childhood and early adolescence has been associated with a 1.15-1.18-fold increased risk of MS in adulthood (5), and overweight and obesity in late adolescence and early adulthood have been associated with an approximate two-fold increased risk of MS in adulthood (6,7). Furthermore, childhood overweight and obesity have been associated with an approximate 1.5- to 3.75-fold increased odds of pediatric-onset MS, depending on the extent of overweight or obesity (8). In addition, recent Mendelian randomization (MR) analyses have demonstrated support for a causal association between body mass index (BMI) and MS, whereby an increase in BMI by approximately 5 kg/m^2 increased the odds of MS by 40% (9). However, the underlying biological mechanisms linking BMI to MS are unclear.

Obesity is associated with chronic, mild inflammation characterized by abnormal cytokine production and increased pro-inflammatory signaling. Adipose tissue is known to produce cytokines, known as adipokines; however, the relative contribution of adipocyte-derived cytokines to the inflammatory state in obesity is unknown (10).

Interestingly, adiponectin, an adipokine with known anti-inflammatory properties in both the innate and adaptive arms of the immune system (10), is reduced in overweight and obese individuals (11,12), and is negatively correlated with BMI (11).

In light of these findings, animal and human studies alike have been undertaken to better understand adiponectin's role in MS etiology and treatment. Results from studies using experimental autoimmune encephalomyelitis models of MS are suggestive of a protective role for adiponectin in rodents (13,14). However, findings from studies in clinical populations are diverse. One study showed reduced levels of this adipokine in peripheral blood of MS patients following acute relapse (15), while others demonstrate elevated adiponectin in peripheral blood and CSF of patients in remission (16,17), or unaltered adiponectin in newly diagnosed MS patients (18).

Observational studies, such as those described above, represent an important step in the identification of risk factors in disease. Randomized control trials (RCTs) and/or MR studies can help to clarify the roles of identified risk factors in disease outcome, as findings of observational studies may be biased due to residual confounding and/or reverse causation. Indeed, numerous RCTs and MR studies have provided strong evidence for the presence of bias in previously reported observational associations (many examples reviewed in 19). However, these types of studies can also validate observational associations through demonstration of causality (also reviewed in 19). One such MR study (9) suggested that previously reported observational associations between body weight and MS (e.g. 5-8) are not likely biased due to confounding or reverse

causation. Nonetheless, no study to date has provided such evidence for the reported observational association between adiponectin level and MS. Confounding due to reverse causation is of particular concern in epidemiological studies of MS, as timing of disease onset is unknown. Therefore the nature of adiponectin's role in MS etiology therefore merits further investigation.

In the absence of experimental studies investigating adiponectin's role in MS clinical populations, MR studies can be conducted to evaluate adiponectin's role in disease outcome in a manner that allows for causal inference. This approach is conceptually similar to a randomized control trial, where instead of randomization to a pharmaceutical intervention, individuals in the population are naturally randomized at conception to varying levels of an exposure (e.g. adiponectin level) due to genetic variation.

MR is a technique which uses genetic variants strongly associated with an exposure (e.g. adiponectin level) to estimate the exposure's effect on disease risk (e.g. MS) (20). Since genetic variants are randomly allocated at meiosis, they are not influenced by confounding factors that may bias observational associations, except confounding by ancestry. Further, reverse causation is overcome since allelic randomization always precedes MS onset.

To better understand whether adiponectin levels may influence risk of MS, we undertook an MR study of adiponectin on MS risk using a two-sample MR design, deriving the effects of single nucleotide polymorphisms (SNPs) on adiponectin and MS risk from the largest adiponectin and MS samples available to date: the ADIPOGen Consortium (N =

45,891) (21), the International Multiple Sclerosis Genetics Consortium (IMSGC, n_{cases} = 14,498 / $n_{controls}$ = 24,091) (22), and the IMSGC/Wellcome Trust Case Control Consortium 2 (IMSGC/WTCCC2, n_{cases} = 9,772 / $n_{controls}$ = 17,376) (23).

Methods

SNP Selection, Effect Sizes, and Data Sources

Genome-wide significant ($p < 5 \times 10^{-8}$) genetic variants associated with adiponectin levels were obtained from ADIPOGen (21). For this study, we limited our selection of SNPs and summary statistics to those that achieved genome-wide significance in the European sex-combined discovery phase analyses or joint analyses ($30{,}708 \leq n \leq 38{,}276$). The effect of each SNP on natural-log-transformed adiponectin levels was adjusted for age, sex, BMI, the principle components of ancestry, study site (where appropriate), and family structure in cohorts with family members (21). Corresponding effect estimates of the adiponectin-associated SNPs on risk of MS were obtained first from the IMSGC Immunochip study, the largest genetic association study for MS (14,498 cases and 24,091 controls) (22), and then from the second largest study, the IMSGC/WTCCC2 (9,772 cases and 17,376 controls) (23), if an adiponectin-associated SNP was not ascertained in the IMSGC Immunochip study. We have previously used these datasets to explore the effects of BMI and vitamin D on risk of MS (9, 24). If summary statistics were not available for an index SNP in either study, a highly correlated proxy ($r^2 > 0.8$) was selected first from the Immunochip study and then from the IMSGC/WTCCC2 study, if the former was unavailable. Linkage disequilibrium (LD) for proxies was measured using UK10K samples (n = 3,781) (25).

SNP Validation

Linkage disequilibrium assessment. MR studies require that the SNPs not be in LD, since strong correlations between selected SNPs may bias results (20). To ensure that the adiponectin-associated SNPs met this requirement, LD was measured between all selected SNPs using European samples from the UK10K project using PLINK software version 1.90 (25). SNPs were excluded from analyses if their measured LD was $r^2 > 0.05$.

Pleiotropy assessment. MR analyses assume that the SNPs influence the outcome (MS) solely through the exposure of interest (adiponectin). To assess for the presence of pleiotropy, MR-Egger regression was performed as previously described (27). This approach is based on Egger regression, which has been used to examine publication bias in the meta-analysis literature (28). In brief, the SNP's effect upon the exposure variable is plotted against its effect upon the outcome, where an intercept distinct from the origin provides evidence for pleiotropic effects. Funnel plots can also be used for visual inspection of symmetry. In addition, a systematic PubMed literature search was conducted to investigate possible pleiotropic mechanisms of the selected SNPs on MS, using a previously described method (24) (**S1 Methods**). Last, pleiotropy was assessed by examining only the SNP at *ADIPOQ*, which encodes adiponectin. Pleiotropy is less likely to influence results at this locus, since it is likely that genetic variation at *ADIPOQ* influences adiponectin levels directly (29).

Population Stratification. To reduce this potential source of bias, selected SNPs and summary statistics for both adiponectin and MS were obtained from analyses involving individuals of European descent only. In addition, a literature search was conducted to

investigate potential residual population stratification that may exist among European subgroups with respect to adiponectin levels (30). To the best of our knowledge, no epidemiological studies have investigated adiponectin levels across European subgroups; therefore, mean adiponectin serum concentrations from the ADIPOGen European cohorts were compared to investigate potential differences in population adiponectin levels across Europe. A Shapiro-Wilks test was used to assess normality of mean adiponectin concentration for the following countries: United Kingdom, United States, Netherlands, Germany, Italy, and Finland. Analysis of variance (ANOVA) was then performed to investigate potential differences in adiponectin concentrations across these countries. Shapiro-Wilks test and ANOVA were performed using Graphpad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA).

Mendelian Randomization Estimates

In this previously described two-sample MR study design (24,31) where independent SNPs evaluate the association of exposure to genetically altered adiponectin levels with MS risk, MR estimates were obtained by weighting each of the adiponectin-associated SNPs by the magnitude of its effect upon natural-log-transformed adiponectin level. The individual estimates were then meta-analysed using a fixed-effects model to obtain a summary measure for the effect of genetically increased adiponectin on risk of MS.

Sensitivity Analyses

If a given SNP violated any of the underlying assumptions of MR, MR estimates were re-calculated excluding that SNP. Further sensitivity analyses were undertaken using (1)

only the lead SNP from ADIPOGen, located near the adiponectin-encoding gene *ADIPOQ*, to reduce potential bias from pleiotropy (29); and (2) only the SNPs genotyped in both ADIPOGen and either of the MS studies, to reduce potential bias from random error introduced by use of proxy SNPs

All statistical analyses were performed using R version 3.2.2 software (32) unless otherwise noted.

Results

SNP Selection

ADIPOGen identified 12 SNPs as genome-wide significant ($p < 5 \times 10^{-8}$) for adiponectin level in European populations (21). Of these, none were genotyped directly in the Immunochip study; however four were found in the IMSGC/WTCCC2 GWAS: rs1108842 (within *GNL3*), rs12922394 (within *CDH13*), rs1597466 (near *TSC22D2*), and rs2925979 (within *CMIP*) (**Table 1**). Proxies ($r^2 > 0.80$) were identified for 6 of the 8 remaining SNPs: one from the IMSGC Immunochip study (rs6810075, near the adiponectin-encoding gene *ADIPOQ*), and five from the IMSGC/WTCCC2 GWAS (rs2980879, near *TRIB1*; rs601339, near *GPR109A*; rs7133378, within *DNAH10*; rs7955516, near *PDE3A*; and rs3001032, near *LYPLAL1*) (**Table 1**). Therefore, 10 of the 12 ADIPOGen SNPs were selected for this MR study. None of the ten adiponectin increasing alleles were significantly associated with MS risk, accounting for multiple testing (all $p > 0.05/10 = 0.005$, **Table 1** and **Figure 1**).

SNP Validation

Linkage disequilibrium. None of the 10 adiponectin-associated SNPs were found to be in LD (all pairwise $r^2 < 0.05$) in the UK10K European samples (25).

Pleiotropy. MR-Egger regression analyses suggested that pleiotropy did not greatly influence the results of the MR analyses (Egger intercept $p = 0.21$; 95% CI: -0.015-0.058). Additionally, a literature review failed to unearth pleiotropic mechanisms for any of the investigated SNPs, with the exception of rs12922394. This SNP is located within an intron of the *CDH13* gene, which encodes T-cadherin, a protein known to bind both high molecular weight (HMW) adiponectin and low-density lipoprotein (LDL). It is thought that T-cadherin might function as a receptor for both these ligands (33). Numerous epidemiological studies have demonstrated associations between elevated serum LDL and MS disease progression, as well as adverse clinical and MRI outcomes (34). Based on these findings, the possibility that CDH13 functions independently of adiponectin to produce MS phenotypes could not be eliminated; therefore, sensitivity analyses were undertaken to exclude rs12922394 from MR analyses.

Population Stratification. A one-way ANOVA revealed that serum log-transformed adiponectin concentrations did not differ across the European subpopulations interrogated in ADIPOGen ($F_{(5,17)} = 1.27$, $p = 0.32$).

Mendelian Randomization Estimates

Employing a fixed-effects model including all ten adiponectin-altering alleles revealed that a one-unit increase in natural-log-transformed adiponectin, which corresponds to a 2-standard deviation (SD) change on the absolute scale, was not associated with a clear

effect on the odds of MS (OR = 0.93; 95% CI: 0.66-1.33; p = 0.61) (**Figure 1**). The I^2 estimate of heterogeneity was 0%, suggesting no heterogeneity of effect. Sensitivity analyses excluding rs12922394 (*CDH13*) for possible pleiotropic effects did not influence these results (OR = 0.93; 95% CI: 0.64-1.37; p = 0.72). Analysis of the *ADIPOQ* variant rs6810075 alone revealed that a one-unit increase of natural-log-transformed adiponectin did not alter the odds of MS (OR: 0.60; 95% CI: 0.34-1.07; p = 0.08). Analysis of the pooled non-proxy SNPs rs1108842, rs12922394, rs1597466 and, rs2925979, revealed no evidence of an association with MS risk (OR = 1.06; 95% CI = 0.60, 1.88).

Discussion

In this MR study investigating the role of adiponectin level upon MS risk, we have demonstrated that a large (2-SD), lifelong genetic increase in adiponectin level was not associated with a clinically-relevant change in the odds of MS. This finding does not support a substantial role for adiponectin in the causal pathway of MS; however, given the wide confidence interval, a small protective or detrimental effect of adiponectin in MS cannot be definitively ruled out, and further studies will be necessary to more clearly ascertain adiponectin's role. Notwithstanding, the present study suggests that a substantial, lifelong alteration in adiponectin levels would be necessary to influence the risk of disease, if adiponectin indeed plays a causal role therein.

Observational studies aiming to shed light on the clinical relevance of adiponectin levels in MS have yielded variable results (15-18,35). Observational studies such as these are

susceptible to bias due to residual confounding, in addition to a number of other factors that may bias observational studies. While the potentially confounding effects of BMI were accounted for in all of these studies, there are several related, physiological effects which were not likely not controlled for through the use of BMI as a measure of obesity, and which could have influenced the reported associations. For example, differences in adipose tissue amount and location can influence adiponectin concentrations, as production of adiponectin is differentially regulated in visceral and subcutaneous adipocytes (36). These differences in adipose distribution are not accounted for in BMI calculations. Differential clearance through the liver could also influence measurements of adiponectin in such studies (36). One strength of the present study is that it utilizes a method of analysis which largely overcomes confounding, due to the random assortment of alleles at conception.

As the present study assessed the association between lifelong genetically-increased adiponectin levels and the odds of development of MS, the findings reported here suggest that adiponectin is not an ideal preventative treatment target for MS. Adiponectin's therapeutic role in MS following disease onset, on the other hand, cannot be ascertained based on the present findings. Interestingly, two of the adiponectin-modulating SNPs investigated in this study (rs601339 and rs7955516) are located near genes implicated in the both the preventative and therapeutic treatment of MS (*GPR109A* (37) and PDE3A (38), respectively). In addition, adiponectin treatment following EAE induction in rodents has been shown to attenuate the clinical course of EAE, findings suggestive of a potential therapeutic role of adiponectin in MS following disease onset (13).

Observational studies (5-8) and MR analyses (9) have indicated that increased body weight and BMI render individuals more susceptible to MS. As an adipokine with anti-inflammatory properties and which is negatively correlated with BMI, adiponectin is a biological candidate of interest in the investigation of the underlying causal pathway of MS. While the present findings cannot rule out the possibility of a protective or detrimental role for adiponectin in MS etiology, they suggest that adiponectin's role in the causal pathway of this disease is likely to be small. Further studies will be necessary to ascertain which biological factors drive the causal association between BMI and MS.

The present study has important limitations. The possibility of residual pleiotropy biasing our estimates remains, despite the sensitivity analyses conducted. MR-Egger results can be biased when the effect on pleiotropic pathways is proportional to its effect on adiponectin level. Interestingly, genetic variation at adiponectin-encoding gene *ADIPOQ* was marginally associated with risk of MS ($p = 0.08$), and variation at this locus is less likely to influence MS risk independently of adiponectin than other the SNPs investigated. In addition, it is impossible, using current methods, to directly assess the extent to which canalization, or developmental compensation, may have influenced our results. While variation in adiponectin level explained by ADIPOGen SNPs is relatively high (~5%), MR relies upon the assumption of linear dose-response effects, which may not be suitable. It is also possible that subtle population stratification of adiponectin levels across Europe biased our results. Yet, no differences in adiponectin level across European populations in ADIPOGen, a consortium measuring adiponectin levels in 26

European or European-descent cohorts, were detected. Finally, as with any null finding, the width of the 95% confidence intervals gives a sense of what effect sizes can be excluded, given the large (2-SD) genetic increase in adiponectin levels.

In conclusion, using data from the largest genetic consortia for adiponectin and MS, we find that lifelong exposure to a substantially (2-SD) genetically-elevated adiponectin level has no clinically-relevant effects on MS susceptibility in individuals of European descent. Adiponectin is therefore not likely to be an ideal candidate target for MS prevention; however, its therapeutic potential for MS following disease onset remains to be determined. Additional studies will be necessary to ascertain which biological factors drive the causal association between body weight and MS.

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ADIPOGen data is publicly available and can be accessed at http://www.mcgill.ca/genepi/adipogen-consortium. IMSGC data is publicly available and can be accessed at https://www.immunobase.org/. WTCCC2 data is available by application only.

Conflict of Interest

The authors declare that there is no conflict of interest.

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